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## The Number of Osteoblast and Osteoclast during Orthodontic Tooth Movement after Preconditioned Gingiva Mesenchymal Stem Cell Allogeneic Transplantation *in vivo*

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### Abstract

Malocclusion can affect the quality of life related to oral health. A novel technique to expedite orthodontic tooth movement (OTM) in order to minimize treatment length and the side effects. Under orthodontic force, MSCs transplantation might hypothetically accelerate the bone remodeling process, resulting in OTM acceleration.

To investigate the number of osteoclasts and osteoblasts in the tension side during OTM after transplantation of normoxic or hypoxic preconditioned allogeneic gingival mesenchymal stem cells *in vivo*.

The OTM animal model was 48 male rabbits (*Oryctolagus cuniculus*) aged 6 months with body weight about 3-4 kg. There were 4 experimental groups in this study: control negative group (C-): injected with PBS without OTM, positive control group (C+): 50g OTM with 20µL PBS injection, treatment group 1 (T1): 50g OTM with 20µL GMSCs normoxia in PBS, treatment group 2 (T2): 50g OTM with 20µL GMSCs hypoxia in PBS. The injection was done in afflicted mandibular gingiva using µL small needle syringe with local infiltration technique. Every sample then was sacrificed after day 7, 14, and 28 respectively. The number of osteoblasts and osteoclasts in the alveolar bone during OTM was determined by hematoxylin and eosin staining.

In T2 group, osteoblast was significantly enhanced on day 14 and 28 but not osteoclast number during OTM. There was significant difference in osteoclast and osteoblast number between groups ( $p < 0.05$ )

GMSCs hypoxia preconditioned escalate osteoblast number but not decrease the osteoclast number in the tension side during orthodontic tooth movement *in vivo*.

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### Introduction

Malocclusion, or crowded, irregular, and prominent teeth, has been an issue for many people since ancient times, and efforts to treat it

have been attempted since at least 1000 BC. Malocclusion is a condition in which the teeth of the upper and lower jaws are positioned improperly. Malocclusion is also an indication of a discordant connection between the jaw and teeth as a result of their shape or size.<sup>1</sup>

Angle's categorization has been utilized by the worldwide community to examine the mesio-distal connections of the dental arches since 1907. It serves as the foundation for determining whether or not a malocclusion in the

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sagittal dimension requires orthodontic treatment. The examination of the occlusal anomaly of setting (centering or guiding) and the category of occlusion (functional occlusion, functional malocclusion, or pathogenic malocclusion), ie its probable pathogenic influence on orofacial structures, must complete the kind of abnormalities (skeletal and dental).<sup>2</sup> Malocclusions are now considered the third most common oral health condition by the World Health Organization, behind dental caries and periodontal diseases.<sup>3</sup>

The frequency of dental and oral health is still very high in Indonesia. Dental and oral diseases impact 57.6 percent of the population, according to the findings of the 2018 National Basic Health Research. Malocclusion is one of the remaining dental and oral disorders in Indonesian population.<sup>4</sup> Malocclusion is one of the dental issues that ranks third in terms of dental and oral health concerns in Indonesia, following dental caries and periodontal disease, with an estimated incidence of around 80%. The high percentage is due to a lack of understanding about dental care.<sup>5</sup>

Malocclusion can affect the quality of life related to oral health (oral health related to quality of life / OHR-QOL). malocclusion is accompanied by a significant increase in overjet related to complaints in the oral cavity, functional limitations of Stomatognathic, and social welfare. Inner bite is also significantly related to oral complaints and functional limitations of Stomatognathic.<sup>6</sup> The socioeconomic situations and aesthetic implications of malocclusion have a significant impact on the quality of life of teenagers.<sup>7</sup>

Orthodontic tooth movement (OTM) is defined as a biological response to a change in the physiological balance of the dentofacial complex induced by an applied external force to treat malocclusion.<sup>2</sup> OTM occurs in the force direction, with bone resorption in the compression area and bone apposition in the tensile area of the periodontal ligament.<sup>8</sup> OTM is induced by tissue remodeling around the tooth root as a result of force application. Remodeling requires the existence of cells capable of absorbing and producing the extracellular matrix of the periodontal ligament and alveolar bone. When orthodontic stresses are applied, the stressed side's periodontal ligament constriction is followed by alveolar bone loss owing to

osteoclast activity. On the other hand, osteoblasts appoint bone on the tensile side.<sup>9</sup>

As modern dentistry evolves, the necessity for quicker orthodontic care and the investigation of methods to deliver the finest form of patient care becomes increasingly desirable. A multitude of minimally invasive surgical and non-surgical treatment options have been developed as a result of extensive study to help speed tooth motions. Although this method shows promise in terms of treatment acceleration, it does not have the ability to improve periodontal outcomes.<sup>10</sup> Surgical treatment time for accelerating the movement of orthodontic treatment depends on the speed of the movement of the tooth which in turn depends on the speed of alveolar bone remodeling. Therefore, it is suspected that an increase in the speed of teeth movement can be achieved by accelerating the biological response of periodontal ligaments and alveolar bones.<sup>11</sup>

Comprehensive orthodontic therapy is time-consuming, but the best data at this time, based on prospective studies done in a university research setting, indicates that it takes less than two years on average to complete.<sup>12</sup> As treatment advances, the risk of root resorption and decalcification increases. However, because the patient desires a much shorter treatment period of only 6-12 months, Orthodontist, the Orthodontic company's material, has performed considerable research to create ways to accelerate therapy. Some orthodontic firms are currently marketing products, materials, and processes that claim to reduce treatment durations in half.<sup>13</sup>

Corticosteroids, vitamin D3, parathyroid hormones, and thyroxine have all been demonstrated in animal studies to stimulate tooth mobility. Human investigations examining the use of prostaglandins in the movement of orthodontic teeth discovered the possibility of increasing teeth movement. As a result, medicines that inhibit the movement of orthodontic teeth, such as aspirin and NSAIDs, have been demonstrated in animal studies to slow the movement of orthodontic teeth.<sup>14</sup> Another method that has been promoted is the use of autologous plasma rich platelets (PRP) as a substitute for cytokines or local medications to recreate the effects induced in the bone following surgery. Platelets, which include growth factors such as Platelet Derived Growth Factor (PDGF) and Transforming Growth Factor (TGF), are the starting point for

soft and hard tissue wound healing (TGF).<sup>13</sup>

Mesenchymal stem cells (MSCs) are self-renewing cells that can develop into a variety of cells under the right conditions.<sup>15</sup> MSCs have been found in muscles, dermis, bone marrow, adipose tissue, periosteum, blood, umbilical cord, synovial membrane, and teeth.<sup>16,17</sup> Some of these sources are easily accessible in orthodontic since excision of oldest teeth, permanent premolar teeth, or wisdom teeth is a common intervention in the treatment of malocclusion orthodontic, MSCs sources from teeth may be collected without further morbidity. Several investigations have indicated the ability of mesenchymal stem cells (MSCs) derived from dental pulp, periodontal ligaments, or exfoliated deciduous tooth to differentiate and proliferate.<sup>18-22</sup>

Defects treated with bone marrow-derived mesenchymal stem cells (BM-MSCs) regenerate more faster and had better reepithelization, cellularity, and angiogenesis.<sup>23</sup>

Orthodontists and researchers have been seeking for novel techniques to expedite OTM in order to minimize treatment length, side effects such as pain, discomfort, dental caries, and periodontal disease, as well as iatrogenic damage such as root resorption, teeth becoming non-vital, and adverse effects.<sup>24</sup> During the application of orthodontic force, an increase in periodontal ligament (PDL) progenitor cells was observed by pressing the expression of type I (Col-I) collagen expression, while after the forced withdrawal with orthodontic appliance increasing the Col-I expression, indicating that periodontal ligament stem cells (PDLSC) is able to respond to the orthodontic mechanical strength with collagen expressions pressed.<sup>25</sup> The ability of this MSCs can be employed to accelerate OTM in response to orthodontic force. When orthodontic force is applied, tooth movement is halted until the necrosis is eliminated, resulting in clinical symptoms of the lag phase. Under pressure, MSCs transplantation might hypothetically accelerate the process, resulting in OTM acceleration.<sup>26</sup>

Bone remodeling is the removal of old or damaged bones by osteoclasts and the formation of new bones by osteoblasts. Osteoclasts are bone absorbent cells that disintegrate bone via acid secretion and proteolytic enzymes such as Cathepsin K (CTSK), which dissolves collagen and other matrix proteins during bone

resorption.<sup>27-29</sup> Meanwhile, osteoblasts are bone-forming cells that develop from mesenchymal precursors with osteoprogenitor derivatives via transcription factor activation and eventually differentiate into osteocytes.<sup>30,31</sup>

Extracellular proteins produced by osteoblasts include osteocalcin, alkaline phosphatase, and type I collagen, which accounts for more than 90% of bone matrix proteins. Extracellular matrix is first produced as an osteoid, which is then terminalized by the buildup of calcium phosphate in the form of hydroxyapatite.<sup>32</sup> To control cell proliferation, differentiation, and survival, osteoblasts and osteoclasts have direct contact via the interaction of EFNB2-EPHB4, FAS-FASL, and NRP1-SEMA3A. TGF- $\beta$  and IGF-1 are released from the bone matrix by osteoclasts, allowing osteoblasts to initiate bone growth. Despite the fact that numerous variables have been found that influence the interaction of osteoblasts and osteoclasts, relatively little from this route has resulted in therapy authorized for the treatment of bone metabolism.<sup>33</sup> Furthermore, the number of osteoclasts and osteoblasts in the tensile side during OTM following transplantation of normoxic or hypoxic preconditioned allogeneic gingival mesenchymal stem cells in young male rabbit (*Oryctolagus cuniculus*) was investigated.

## Materials and methods

### Ethical Clearance Statement

All techniques in this study were carried out in compliance with the applicable standards and regulations established by the Ethics Committee of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia.

### Experimental Study Design

This study was true experimental laboratory study with post-test only control group design. The sample was selected blind randomly and the sample size was determined using minimal sample size formula. A sample of 48 male rabbits (*Oryctolagus cuniculus*) aged 6 months with body weight ranging from 3-4 kg and declared healthy by physical examination and having a complete dentition and healthy periodontium were carried out by veterinarian. Each group consists of 4 samples. Each rabbit was kept in a cage measuring 60 x 40 x 40 cm and adapted for 1 week before treatment. Each sample was kept in a controlled temperature

room ( $22 \pm 2^{\circ}\text{C}$ ) with a 12-hour dark and light cycle and was able to access feed and drink according to standards. There were 4 experimental groups in this study: control negative group (C-): injected with PBS without OTM, positive control group (C+): OTM with 20 $\mu\text{L}$  PBS injection, treatment group 1 (T1): OTM with 20 $\mu\text{L}$  GMSCs normoxia in PBS, treatment group 1 (T2): OTM with 20 $\mu\text{L}$  GMSCs hypoxia in PBS. The injection was done in afflicted mandibular gingiva using  $\mu\text{L}$  small needle syringe (Terumo, US) with local infiltration technique.

#### **Application of Orthodontic Tooth Movement**

OTM appliances was installed between the incisor and mandibular molars using a 6 mm NiTi close coil spring (American Orthodontics Corp., Sheboygan, WI, USA) to move the molar mesially with 50g mild force of OTM and measured with a tension gauge (American Orthodontics Corp., Sheboygan, WI, USA). 0.07 stainless steel ligature wire (American Orthodontics Corp., Sheboygan, WI, USA) was used to secure the fixed orthodontic device. To decrease discomfort in the animals during treatment, an anesthetic was provided intravenously (3 percent pentobarbital sodium, 1 ml/kg).<sup>34</sup> Every sample then was sacrificed through slaughter by means of sharp blade after day 7, 14, and 28 respectively.

#### **Gingiva Mesenchymal Stem Cell culture, sub-culture, confirmation, hypoxia preconditioned and transplantation**

The GMSC isolation process was carried out in accordance with method conducted by Nugraha et al.<sup>35</sup> GMSCs were extracted from the gingiva of a healthy male rabbit (*O. cuniculus*) aged 6 months and weighing 3-5 kg in the lower jaw area using a scalpel and blade. The gingiva was chopped into tiny pieces and grown in Dulbeccos Modified Eagle Medium (DMEM, Life Technologies/GIBCO BRL) with 20% Fetal Bovine Serum (FBS, Biochrom AG, Germany), 5m M L - glutamine (Gibco Invitrogen), 100 U/ml penicillin-G, 100 g/ml streptomycin, and 100 g/ml kanamycin. After three days, the medium was withdrawn in order to eliminate the cell components that were not connected to the dish, and a fresh media was provided. FGF-2 was introduced at this point. After the cells were confluent, they were passaged with 0.05 percent trypsin-EDTA, washed, and cultivated again in 60- or 100-mm tissue culture dishes (Corning).

The confluent cells were re-passed, and the cells were ready for further study. If the cells are not used right away, they should be kept in liquid N<sub>2</sub>. Thawing GMSCs begins with removing the cell vial from the freezer. The vial was immersed in a 37 $^{\circ}\text{C}$  water bath for 1 minute, until the cells started to melt. Make certain that the bottle does not sink and that no water enters the bottle. To sterilize the vial, it is withdrawn from the water bath and wiped with 70% isopropanol or ethanol. Cells were transferred into a 15-ml tube containing 10 ml of Iscove's Modified Dulbecco's Media (IMDM) + 2% Fetal Bovine Serum using a sterile 2 ml pipette (FBS).

The cells were centrifuged and discarded, and the cell pallet was resuspended in an initial volume of IMDM +2 percent FNS and the total cell volume to be cultivated up to the 4th passage according to study needs. GMSCs are MSCs isolated from the free gingival margins of experimental animals and have the capacity to differentiate mesenchymal lineages such as osteogenic differentiation proven by the Alizarin's Red staining procedure positive characterized by mineral deposits.<sup>36</sup> Adaptive GMSCs were passage 4 GMSCs that had been hypoxia preconditioned by treating them in vitro for 24 hours with 100 M Cobalt Chromium (CoCl<sub>2</sub>) as a hypoxia mimicking agent (HMA).<sup>37</sup> After 1 day of PGO, hypoxic GMSCs of 10<sup>6</sup> cells in L in PBS were administered in a single dose with a tiny needle syringe (Terumo, US) using a local infiltration approach into the gingiva of male rabbits (*O. cuniculus*).

#### **Hematoxylin eosin staining to examine osteoblast and osteoclast number**

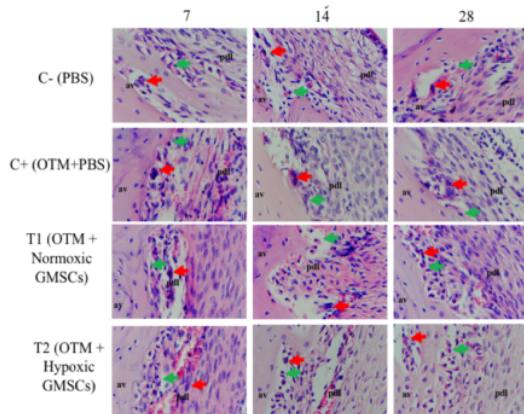
The rabbit's mandibular of each sample was dissected and put in 10% neutral buffer formalin (OneMed, Surabaya, Indonesia) for four days before being decalcified for three months with ethylenediaminetetraacetic acid (EDTA) (Leica, Germany). The number of osteoblasts and osteoclasts in the alveolar bone during OTM was determined by mounting histological slices of samples on glass microscope slides and staining them with hematoxylin and eosin (Sigma Aldrich, St Louis, MO, USA). The number of osteoblasts and osteoclasts from the five images were first accumulated for each rabbit, with the mean of the five sections then calculated under 400x magnifications by means of an Olympus Light Microscope (CX23, Olympus, New York, USA) from five different fields of view.<sup>38,39</sup>

### Statistical analysis

The research data was then summarized and analyzed descriptively and inferentially. A bar chart containing the mean and standard deviation is used to display the data. The data were analyzed using the statistical package for social science (SPSS) version 20.0 for Windows, which included the normality and homogeneity test ( $p > 0.05$ ), the analysis of variance (ANOVA) difference test, and the post-hoc Tukey Honest Significant Different (HSD) with a  $p < 0.05$  significance value (IBM corporation, Illinois, Chicago, US).

### Results

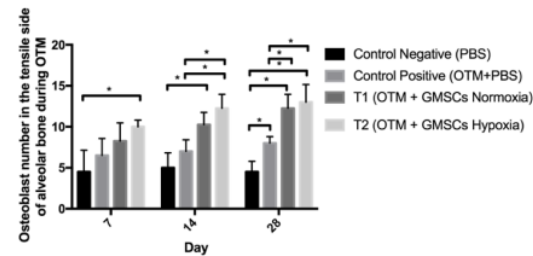
Osteoblasts and osteoclasts were found on histopathological examination in all groups (C-; C+, T1, T2). The morphology of osteoblasts is single-nucleated, round cells located on the surface of the alveolar bone, whereas the morphology of osteoclasts is large, multinucleated cells located on the bone surface accompanied by resorption areas of osteoblasts (green arrows) and osteoclasts (red arrows), seen with a light microscope at 400x magnification (Figure 1).



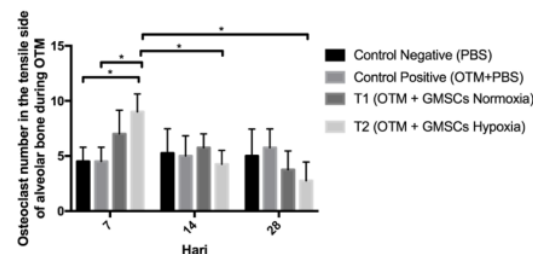
**Figure 1.** Observations on the morphology and quantity of osteoblasts (green arrows) and osteoclasts (red arrows) in alveolar bone using a light microscope at 400x magnification.

Figure 2 depicts the mean and standard deviation (SD) of the number of osteoblasts in each group. On day 28, the highest number of osteoblasts were detected in the treatment group 2 (T2), whereas the lowest number of osteoblasts

were found in the negative control group (C-) on days 7 and 28. The number of osteoblasts differed significantly across the treatment groups and between the observation days. On day 7, there was a substantial difference in the number of osteoblasts between C- and PII. On day 14, the quantity of osteoblasts differed significantly between C- and T2; C+ and T2.



**Figure 2.** Bar chart of mean and standard deviation (SD) of osteoblast counts between groups. \*information: significant at  $p < 0.05$  between groups.



**Figure 3.** Bar chart of mean and standard deviation (SD) of osteoclast counts between groups. \*information: significant at  $p < 0.05$  between groups.

Figure 3 shows the mean and standard deviation (SD) of the number of osteoclasts in each group. In this study, the treatment group 2 (T2) had the most osteoclasts on day 7 and the control group (C-) had the fewest on day 28 after treatment 2 (T2). The number of osteoclasts differed significantly between the days of observation. On day 7, there was a substantial difference in the number of osteoclasts between C- and T2, C+ and T2. There was a substantial difference in the number of osteoclasts between T2 on day 7 and T2 on day 14, and T2 on day 14 and T2 on day 28.

| Group          | Day                     |                            |                           | P       |
|----------------|-------------------------|----------------------------|---------------------------|---------|
|                | 7                       | 14                         | 28                        |         |
|                | Mean ± SD               |                            |                           |         |
| C-             | 4.5 ± 2.65 <sup>a</sup> | 5 ± 1.83 <sup>bc</sup>     | 4.5 ± 1.29 <sup>ab</sup>  | 0.0001* |
| Normality test | 0.689                   | 0.714                      | 0.972                     |         |
| C+             | 6.5 ± 2.08              | 7 ± 1.41 <sup>d</sup>      | 8 ± 0.82 <sup>ef</sup>    |         |
| Normality test | 0.995                   | 0.161                      | 0.683                     |         |
| T1             | 8.25 ± 2.22             | 10.25 ± 1.9 <sup>b</sup>   | 12.25 ± 1.71 <sup>h</sup> |         |
| Normality test | 0.798                   | 0.224                      | 0.850                     |         |
| T2             | 10 ± 0.82 <sup>g</sup>  | 12.25 ± 1.71 <sup>cd</sup> | 13 ± 2.16 <sup>g</sup>    |         |
| Normality test | 0.683                   | 0.850                      | 0.577                     |         |
| p-value        | 0.006*                  |                            |                           |         |

**Table 1.** Description of mean, standard deviation (SD), and test of differences between groups of osteoblast counts.

\* information: significant at p<0.05 between groups and days of observation. <sup>abcdegh</sup> the same superscript showed that there were differences between groups (based on the Tukey HSD test).

| Group          | Day                      |                          |                          | p-value |
|----------------|--------------------------|--------------------------|--------------------------|---------|
|                | 7                        | 14                       | 28                       |         |
|                | Mean ± SD                |                          |                          |         |
| C-             | 4.5 ± 1.29 <sup>a</sup>  | 5.25 ± 2.22              | 5 ± 2.45                 | 0.852   |
| Normality test | 0.972                    | 0.798                    | 0.262                    |         |
| C+             | 4.5 ± 1.29 <sup>b</sup>  | 5 ± 1.82                 | 5.75 ± 1.71              |         |
| Normality test | 0.972                    | 0.714                    | 0.850                    |         |
| T1             | 7 ± 2.16                 | 5.75 ± 1.26              | 3.75 ± 1.71              |         |
| Normality test | 0.577                    | 0.406                    | 0.850                    |         |
| T2             | 9 ± 1.63 <sup>abcd</sup> | 4.25 ± 1.26 <sup>c</sup> | 2.75 ± 1.71 <sup>d</sup> |         |
| Normality test | 0.683                    | 0.406                    | 0.850                    |         |
| p-value        | 0.012*                   |                          |                          |         |

**Table 2.** Description of mean, standard deviation (SD), homogeneity test, normality test and difference test between groups of osteoclast counts.

\* information: significant at p<0.05 between days of observation. <sup>abcdegh</sup> the same superscript showed that there were differences between groups (based on the Tukey HSD test).

Figure 3 shows the mean and standard deviation (SD) of the number of osteoclasts in each group. In this study, the treatment group 2 (T2) had the most osteoclasts on day 7 and the control group (C-) had the fewest on day 28 after treatment 2 (T2). The number of osteoclasts differed significantly between the days of observation. On day 7, there was a substantial difference in the number of osteoclasts between C- and T2, C+ and T2. There was a substantial difference in the number of osteoclasts between T2 on day 7 and T2 on day 14, and T2 on day 14 and T2 on day 28.

### Discussion

The forces created by the OTM that activate the osteoblasts cause alveolar bone apposition on the tension side. MSCs endogen, which are local precursor cells, give rise to osteoblasts. Mature osteoblasts generate osteoid, which is then mineralized in bone. Several enzymes have also been identified as being

involved in the production of tensile alveolar bone. Alkaline phosphatase is another another biochemical marker that may be beneficial during osteoblast activity. MSCs can develop into osteogenic differentiation and promote osteoblast proliferation and differentiation on the pull side of the OTM.<sup>24</sup> Previously reported that injection of moringa oleifera extract at 5%, 10%, and 20% concentrations might enhance the number of osteoblasts on the tensile side during OTM in guinea pigs.<sup>40</sup>

Antioxidant activity can boost osteogenic activity, which in turn boosts osteoblastogenesis. Flavonoids can influence cell activity by promoting TGF- $\beta$  production, whereas TGF- $\beta$  increases osteoblast proliferation and migration. Increased osteoblast proliferation and activation can increase the number of osteoblasts, which raises the expression of osteoprotegerin (OPG).<sup>41</sup> Increased OPG expression influences osteoclast activation because OPG acts as a receptor for receptor activator of nuclear factor kappa-B ligand (RANKL) by blocking the interaction of RANKL and receptor activator of nuclear factor kappa-B (RANK) on the cell membrane of osteoclast precursors.<sup>42</sup> There are several theories and speculations about the use of stem cells and/or cell therapy as companion therapy during OTM treatment. Despande discovered new bone creation that regenerates like bone in normal settings, with appropriate vascularity and lamellar development, following autologous transplantation of osteoblast culture, which was histopathologically analyzed. Autologous cultivated osteoblast cells had the greatest outcomes in terms of adaptability to various adjuncts, bone regeneration quality, and structural and functional appropriateness.<sup>43</sup>

Progenitor cells inside BMSCs give rise to osteoblasts. BMP, a Wnt signaling pathway ligand, as well as fibroblast growth factor (FGF), insulin growth factor (IGF), and TGF- $\beta$ , are biological agents that mediate the proliferation and differentiation of osteoblast precursors, eventually encouraging bone production.<sup>44</sup> The activation of key transcription factors, including runt related transcription factor (RUNX2), as well as the activation of the bone morphogenetic protein (BMP) and Wnt signaling pathways, collectively induce osteogenic differentiation of MSCs and initiate osteoblastogenesis. Activated and matured osteoblasts produce osteogenic genes that encode a variety of proteins and

enzymes, including osteonectin, osteocalcin, ALP, and collagen type 1a (Col1a1), all of which are required for the creation of extracellular organic matrix and bone mineralization.<sup>45-48</sup>

MSCs produced from a given tissue maintain some of the 'memory' and qualities of the original tissue, exhibiting tissue-specific origin attributes defined by the source microenvironment.<sup>49</sup> MSCs were discovered for the first time in the oral cavity in the periodontal ligament.<sup>50</sup> GMSCs were initially discovered to be heterogeneous and multipotent. During cultivation, human GMSCs were discovered to exhibit high proliferation rates, stable morphology, stable karyotype, and telomerase activity.<sup>51</sup> *In vitro* osteogenic potential of GMSCs has been frequently proven.<sup>52,53</sup>

During OTM, many tissue reactions to bone mechanical stresses occur. The canonical Wnt-catenin signaling pathway is activated, resulting in transcriptional activation of a variety of osteogenic genes that promote osteoblast differentiation and maturation, as well as bone production. The Wnt-catenin signaling system may interact with other intracellular signaling pathways induced by prostaglandins, nitric oxide (NO), or BMPs. These interactions aid in the development of bones.<sup>54</sup> The applied force is transmitted through the compressed tissue matrix to local cells in the periodontal ligament and alveolar bone, causing the cells to release pro-inflammatory, angiogenic, and osteogenic agents, triggering the remodeling process of the adjacent alveolar bone and periodontal ligament, allowing tooth movement.<sup>55,56</sup>

The periodontal ligament and alveolar bone can drive enhanced osteogenic gene expression by transforming osteogenic progenitor cells into mature osteoblasts that deposit osteoid and then mineralize it.<sup>55,57</sup> Mechanically produced tensile pressures stimulate endogenous MSCs in alveolar bone tissue, activating the regulated protein kinase ERK 1/2, a member molecule of the mitogen activated protein kinase (MAPK) intracellular signaling cascade. The ERK 1/2 pathway then activates the transcription factor RUNX2, which is a key regulator of osteogenic gene expression throughout the differentiation and maturation of osteogenic precursor cells into osteoblasts that generate ALP, Coll1a1, and osteocalcin.<sup>57,58</sup>

Mechanical tensile pressures can therefore activate the intracellular ERK 1/2-

RUNX2 pathway in endogenous MSCs in bone for osteogenic differentiation and facilitate their development into bone-forming osteoblasts.<sup>59</sup> The MAPK intracellular signaling system also comprises the c-JUN N-terminal kinase (JNK) cascade and the p38 cascade, in addition to the ERK 1/2 cascade. The MAPK pathway, namely the p38 cascade, controls the production of inflammatory cytokines and RANKL by osteoblasts, consequently beginning osteoclastogenesis and promoting bone remodeling in periodontal tissues in response to mechanical tensile pressures.<sup>60</sup>

The degree of the tensile strain on the periodontal tissue, which in the context of orthodontic therapy is defined by the properties of the orthodontic force applied, determines the selective activation of intracellular MAPK signaling pathways, whether via ERK 1/2, JNK, or p38.<sup>61</sup> When a tensile force is applied to the tensile side after 72 hours, it looks identical to after 24 hours, but the increase in osteoblasts is accompanied with new bone production.<sup>57</sup> It was discovered in a previous study that moderate hypoxia (10% O<sub>2</sub>) appears to boost the rate of proliferation of osteoblast-enriched cells *in vitro*. Hyperoxia (90% O<sub>2</sub>), on the other hand, has the opposite effect, as a shift in oxygen tension from low to high leads in a dramatic drop in the rate of osteoblast proliferation. Hypoxia can have a significant impact on bone remodeling.<sup>62</sup> Under these conditions, growth factors and other biologic agents are released from the bone matrix and from squeezed blood vessels, and endogenous MSCs are recruited to bone.<sup>44</sup>

Mechanical stimulation during OTM not only influences bone homeostasis by increasing osteoclastogenesis, but it also influences osteoblastogenesis. Previous research has demonstrated that enhanced osteoblast activity causes upregulation of bone production, which is a frequent impact of OTM on the tensile side.<sup>63</sup>

OTM is primarily mediated by osteoblasts and osteoclasts. Many investigations on osteoclasts and osteoblasts during OTM have been conducted. This pattern of cellular activity that occurs during the differentiation of MSCs into osteoblasts and HSCs into osteoclasts can be used to identify possible indicators linked with OTM. Multinucleated cells called osteoclasts destroy and reabsorb bone. Osteoclasts work with osteoblasts to rebuild bone. Osteoclasts refine their ability to dissolve hydroxyapatite



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crystals and destroy organic bone matrix rich in collagen fibers. Osteoclasts are related to immune cells such as megakaryocytes, granulocytes, monocytes, and macrophages. Despite their shared origin, osteoclasts and macrophages in the immune system both have phagocytic roles in bone, thus the term bone-specific macrophages. In pathological situations, osteoclasts act as immunomodulators by secreting different mediators. Osteoclasts have high levels of tartrate resistant acid phosphatase (TRAP), Cathepsin K, and OPG expression.<sup>24</sup> A recent research revealed that injection of moringa oleifera extract at 5%, 10%, and 20% doses might lower the number of osteoclasts on the tensile side during OTM in guinea pigs.<sup>40</sup> Antioxidant-active biomaterials can lower the number and activity of osteoclasts.<sup>64</sup> MSCs have antioxidant properties because they regulate the expression of heat shock protein (HSP) and High mobility group box 1 protein (HMGB-1). MSCs have also been linked to the production of regulatory factors that control osteoclast growth and bone resorption.<sup>65</sup> MSCs exhibit immunomodulatory and immunosuppressive properties *in vitro* and *in vivo*. In a steroid-induced osteoporosis paradigm, MSC transplantation increases bone matrix synthesis and decreases bone resorption, resulting in an improvement in bone density and structure. MSCs, like other microenvironment-dependent immune cells, have a dual influence on osteoclasts. Preconditioned MSCs implanted *in vivo* may alter bone osteogenesis potential.<sup>66</sup>

MSC-based regenerative treatment is now popular due to its high potential. Several clinical trials have been attempted to use it to treat bone resorption disease. MSCs inhibited the production of osteoclast development transcription factors by decreasing NF- $\kappa$ B and nuclear factor associated T-cell 1 (NFATc1) activity. These findings show that MSC-derived factors limit osteoclast development in RAW264 cells when co-cultured with MSCs, which can also influence osteoclast differentiation via OPG expression. It has been observed that MSCs transplantation was commenced 4 weeks after MSCs transplantation to dramatically accelerate bone regeneration by lowering the number of differentiated osteoclasts. It was discovered that MSCs reduced osteoclast differentiation.<sup>67</sup> This is consistent with the findings of this investigation, which found that preconditioning allogeneic GMSCs transplantation with hypoxia and normoxia can lower the number of osteoclasts on the tension side of the alveolar bone during OTM.

## Conclusions

Based on the outcomes of this study, it can be concluded that GMSCs hypoxia preconditioned escalate osteoblast number but decrease the osteoclast number in the tension side during orthodontic tooth movement. However, more research is urgently needed to identify the molecular marker associated with bone resorption and bone apposition during orthodontic tooth movement *in vivo*.

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## Declaration of Interest

The authors declare there is no conflict of interest in this study

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